Supplementary Methods

Multi-tissue northern blot analysis. The human multiple tissue northern (MTN) blot (Clontech), which has equal amounts of total RNA loaded in each lane, was hybridized with a random primer-labeled probe for *G0S2*.

Gene expression levels upon TNFα treatment. PFF cells were treated with TNFα (25 ng/ml) and at various time points RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. 2 μg of mRNA was used to synthesize cDNA using an oligo(dT) primer and Superscript cDNA synthesis kit (Invitrogen). Gene expression levels were analyzed by quantitative RT-PCR using Platinum SYBR Green qPCR SuperMix-UDG with Rox (Invitrogen) and the following primers: *G0S2* (forward, 5'-GGCCTGATGGAGACTGTGTG-3' and reverse, 5' CTTGCTTCTGGAGAGCCTGT-3), *Bcl-XL* (forward, 5'-GGAGCTGGTGGTTGACTTTC-3' and reverse, 5'-CTCCGATTCAGTCCCTTCT G-3'), *cIAP1* (forward, 5'-GCATTTTCCCAACTGTCCAT-3' and reverse, 5'-CATTCGAGCTGCAT GTGTCT-3'), *cIAP2* (forward, 5'-CAACAGATCTGGCAAAAGCA-3' and reverse, 5'-TTGCT CAATTTTCCACCACA-3'), and *GAPDH* (forward, 5'-TGCACCACCAACTGCTTAGC -3' and reverse, 5'-GGCATGGACTGTGGTCATGAG-3'). *GAPDH* provided an internal normalization control, and the expression level obtained at time zero was assigned as 1.

Immunofluorescence and fluorescence microscopy. H1299 cells were infected with Ad-G0S2 or Ad-G0S2(R57A, D58A) and 24 h later, cells were fixed in 4% paraformaldehyde (in PBS), permeabilized in 0.5% TritonX-100 (in PBS) and stained with an α-HA monoclonal antibody (Sigma) followed by α-mouse Ig Texas Red-conjugated secondary antibody (Jackson Laboratories). Mitochondria were visualized using Mitotracker (Molecular Probes/Invitrogen). Cells were visualized with a Zeiss Axiophot2 fluorescence microscope using Axiovision 3.1 software.

RNA interference. PFF cells were stably transduced with a G0S2 shRNA (oligo ID V2LHS-114719) or a non-silencing shRNA (5'-TCTCGCTTGGGCGAGAGTAAG-3'), obtained from Open Biosystems.

Oncomine database searches. Microarray datasets were accessed using the Oncomine Cancer Profiling Database (www.oncomine.org) (1). The brain cancer dataset includes 23 normal brain samples from epilepsy patients and 50 oligodendroglioma samples (2); the breast cancer dataset includes 9 normal breast samples and 47 breast carcinoma samples (3); the myeloma dataset includes 22 normal bone marrow samples and 12 smoldering multiple myeloma samples (4); the ovarian cancer dataset includes 4 normal ovary samples and 37 ovarian endometrioid adenocarcinoma samples (5); and the renal cancer dataset includes 10 normal kidney tissue samples and 10 clear cell renal cell carcinoma samples (6). Box plots depicting gene expression in each sample, as well as a Student's T-test giving a P-value for the comparison of candidate gene expression between the groups, were obtained directly through the Oncomine 3.0 software.

Oncogene-induced transformation assays.

Mouse embryonic fibroblasts (MEFs) at passage 4 from C57BL/6 mice (a kind gift from Emmanuel Petroulakis) were seeded at $2x10^5$ cells per well in a 6-well plate for 24 h. shRNA constructs (the G0S2 vector TRCN0000126076 or non-silencing shRNA vector SHCOO2; Open Biosystems) were transfected into 293 T/17 packaging cells, and the resulting lentiviral particles were used to infect MEFs in the presence of 8 μg/ml polybrene. Twenty-four hours later, MEFs were infected with a retrovirus carrying either empty vector (LPC) or the oncogenes E1A and Ras (kindly provided by Nahum Sonenberg), and 48 h later, cells were transferred to a selection media containing 2 μg/ml puromycin. When cell transformation was visible (3 weeks post-infection), cells were fixed with methanol and stained with methylene blue to visualize transformed foci.

References

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